

NO:65), MUC1¹⁶⁷⁻¹⁷⁵ (ALGSTAPPV; SEQ ID NO:3) or MUC1⁷⁹⁻⁸⁷ (TLAPATEPA; SEQ ID NO:5). CTL bulk cultures were tested against Jurkat-A*0201K^b cells loaded with the cognate peptide (filled triangles) or irrelevant influenza matrix control peptide (open circles). Three representative graphs for each peptide are shown. The vertical axis shows % specific lysis.

Please replace Table 2 at page 36 as follows:

Allele tested	elution pH	Reference Polypeptide			Positive Control Polypeptide (SEQ ID NO:)	B-EBV Line	HLA Type
		sequence (SEQ ID NO:) <i>(origin)</i>	Conc. pmol/μl	Final Conc. nM			
A1	pH 3.1	YLEPAC*AKY (68)	183	150	CTELKLSDY (74) <i>(Influenza NP 44-52)</i>	MAR	A01, A02, B08, B27, C01, C07
A2	pH 3.1	FLPSDC*FPSV (69) <i>(HBV core 18-27)</i>	250	150	GILGFVFTL (75) <i>(Influenza matrix 58-66)</i>	JY	A02, B07, C07
A3	pH 3	KVFPC*ALINK (70)	28 and 20	150	QVPLRPMTYK (76) <i>(HIV nef 73-82)</i>	FRE	A03, A24, B35, B08, C04, C07
A11	pH 3	KVFPC*ALINK (70)	28 and 20	150		BVR	A11, B35, C04
A24	pH 3.1	RYLKC*QQLL (71) <i>(HIV gp41 583-591)</i>	66 and 20	150	AYGLDFYIL (77) <i>(melanoma p15 10-18)</i>	YT2	A24, B54, C01
B7	pH 3.1	APAPAPC*WPL (72) <i>(human p53 84-93)</i>	29 and 20	150	RPIFIRRL (78) <i>(EBNA-3A 379-387)</i>	JY	A02, B07, C07
B8	pH 3.1	FLRGRAC*GI (73) <i>(EBNA-3 339-347)</i>	20	150	YLKDQQQLL (79) <i>(HIV gp41 591-598)</i>	MAR	A01, A02, B08, B27, C01, C07

Please replace the first paragraph at page 41 as follows:

C3
Peptide binding to HLA-A*0201 was analysed using HLA-A*0201⁺ B lymphoblastoid JY cells in a semi-quantitative competition assay (van der Burg et al. (J. Immunol. 156 (1996), 3308-3314)). The assay is based on competitive binding of two peptides to acid stripped MHC class I molecules on a B cell line (JY). A test peptide competes with a fluorescently labeled reference peptide for the empty class I molecules on the cell surface. Mild-acid-treated JY cells were incubated with 150nM fluorescein (FL)-labeled reference peptide FLPSDC(-FL)FPSV (SEQ ID NO: 69) and with several concentrations of competitor peptide for 24 hours at 37°C in the presence of 1.0µg/ml β2-microglobulin. Subsequently, the cells were washed, fixed with paraformaldehyde and analysed by flow cytometry. The mean fluorescence (MF) obtained in the absence of competitor peptide was regarded as maximal binding and equated to 0%; the MF obtained without reference peptide was equated to 100% inhibition. The percentage inhibition was calculated using the formula:

C4
Please replace the table at page 42 as follows:

Peptide Position	Amino Acid Sequence	Motif Score *	IC ₅₀ mM/ml
Flu Matrix ⁵⁸⁻⁶⁶	GILGVVF ^L (SEQ ID NO: 75)	54	<5
MUC1 ²⁶⁴⁻²⁷²	FLSFHISNL (SEQ ID NO: 4)	59	3-5
MUC1 ⁴⁶⁰⁻⁴⁶⁸	SLSYTNPAV (SEQ ID NO: 6)	62	5-10
MUC1 ¹³⁻²¹	LLLTVLTVV (SEQ ID NO: 65)	63	6-10
MUC1 ¹⁶⁷⁻¹⁷⁵	ALGSTAPPV (SEQ ID NO: 3)	64	10
MUC1 ⁷⁹⁻⁸⁷	TLAPATEPA (SEQ ID NO: 5)	58	10-15
MUC1 ¹⁰⁷⁻¹¹⁵	ALGSTTPPA (SEQ ID NO: 66)	56	25

C5
Please replace the last full paragraph at page 42 as follows:

Transgenic mice expressing the chimeric protein A*0201K^b (Vitiello et al., loc. cit.) were immunised subcutaneously in the base of the tail with 100µg of MUC1-derived peptide and 140µg of H-2 I-A^b-restricted HBV core antigen-derived T helper epitope (amino acid

sequence; TPPAYRPPNAPIL; SEQ ID NO:80) (Milich et al., Proc. Natl. Acad. Sci. USA 85 (1988), 1610-1614) emulsified in a 1:1 ratio with Incomplete Freund's Adjuvant (IFA) in a total volume of 200 µl. After a minimum of two weeks, the mice were boosted using the same protocol.

Please replace the first two full paragraphs at page 45 as follows:

To test whether the HLA-A*0201 binding peptides that were previously identified could protect A2K^b transgenic mice (Vitiello et al., loc. cit.) against subsequent tumour challenge with B16-MUC1-A2K^b, groups of 6-8 animals were immunised with 100µg of peptide in IFA in the presence of 140µg of the H-2 I-A^b-restricted HBV core antigen-derived T helper epitope (128-140; sequence TPPAYRPPNAPIL; SEQ ID NO:80)(Milich et al., loc. cit.), on day -28, boosted on day -14 and challenged with 5x10⁵ B16-MUC1-A2K^b cells on day 0. Control mice were given IFA or PBS. A measurable tumour was defined as having a volume greater than 36 mm³.

C b
Results from these experiments are shown in the tables below in the form of the percentage of mice surviving at a given day. For experiments 2 and 3, results of using a vaccinia construct that expresses MUC1 (VV-MUC1) are also shown. In other experiments, immunising with MUC1¹⁶⁷⁻¹⁷⁵ and boosting with MUC1⁷⁹⁻⁸⁷, or immunising with MUC1⁷⁹⁻⁸⁷ and boosting with MUC1¹⁶⁷⁻¹⁷⁵, gave a percentage survival of between 60 and 70% at day 30. Experiment 3 shows results from an experiment in which the mice were inoculated with 8x10⁵ A2K^b dendritic cells (DC) which had been pulsed with the peptides.

IN THE SEQUENCE LISTING:

Please replace the previously submitted paper copy of the Sequence Listing with the attached substitute Sequence Listing.